AD_____

Award Number: W81XWH-04-1-0038

TITLE: Use of Synthetic Nerve Grafts to Restore Cavernous Nerve Function Following Prostate Cancer Surgery: In Vitro and In Vivo Studies

PRINCIPAL INVESTIGATOR: Badrinath R. Konety, M.D.

CONTRACTING ORGANIZATION: University of California San Francisco
San Francisco, CA 94143

REPORT DATE: September 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
31-03-2007	Annual	1 Mar 2006-28 Feb 2007

4. TITLE AND SUBTITLE

Use of synthetic nerve grafts to restore cavernous nerve function following prostate cancer surgery: in vitro and in vivo studies

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0038

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Badrinath R. Konety, M.D.

E-Mail: bkonety@urology.ucsf.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California San Francisco
San Francisco, CA 94143

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The hypothesis of this proposal is that interposition of micropatterned biodegradable polymer based nerve grafts to replace resected or damaged cavernous nerves can facilitate return of erectile function by engendering directional nerve growth in an animal model. Micropatterned grafts have been produced using a biodegradable polymer (PDFA). The groove characteristics obtained by micropatterning will be optimized to allow maximal directional neurite growth have been optimized. The effect of laminin +/- polylysine, Schwann cell and neuronal stem cells on neurite growth are being investigated. Tubulized sheets of the polymer with and without these factors/cells have been used to microsurgically replace resected cavernous nerve in male Sprague-Dawley rats. Control groups consisting of ungrafted animals as well as those grafted with native genitofemoral nerve have been generated. After an interval of 2 months, we have attempted to observed the restoration of physiologic function of the cavernous nerve by electrical stimulation of the nerve and/or pelvic ganglion. We are in the process of analyzing the tissue sections of the grafts that have been harvested to look for nNos expression and fluorogold staining which would indicate re-establishment of nerve integrity since that would be required to allow travel of fluorogold injected into the penile tissue to the pelvic ganglion.

15. SUBJECT TERMS
Radical prostatectomy, impotence, nerve grafts

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	20	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

INTRODUCTION

Adenocarcinoma of the prostate has become the most common malignancy in American males. In 2003 an estimated 220,900 men will be diagnosed with the disease and 28,900 will die of it[1]. At 50 years of age or older, the estimated probability of developing clinically apparent carcinoma is 9.6% and 11.4% for white and black men, respectively[2,3]. Curative options for prostate cancer include radiation therapy and radical prostatectomy. Radical prostatectomy is considered by many to be the treatment of choice for patients with localized cancer of the prostate and offers these patients the greatest chance for long term survival[4-9]. The major side effects of prostatectomy are impotence and incontinence. Prior to the advent of nerve-sparing surgical techniques, it is estimated that less than 10% of patients who underwent radical prostatectomy recovered erectile function postoperatively and urinary incontinence rates as high as 87% were reported [10]. Since the original description of the importance of the peri-prostatic neurovascular bundle in preserving erectile function by Walsh and Donker [11] the anatomic nerve sparing operation has become the standard approach in most cases. This modified surgical approach significantly reduces the impotence rates in these patients[4,5,12,13]. There is some evidence that the neurovascular bundles may contribute to the preservation of continence as well by maintaining autonomic innervation to the smooth muscle in the distal urethra and the somatic motor supply to the striated sphincter[7,12,13]. The neurovascular bundles are located within the lateral prostatic fascia dorsolateral to the prostate and lateral to the membranous urethra. They are susceptible to injury during the standard radical prostatectomy performed either by the retropubic or the perineal approach. To ensure an adequate cancer operation in cases where the nerves are adherent to the prostate and there is increased suspicion of extraprostatic extension of tumor, it would be necessary to sacrifice the neurovascular bundle on that side. The potency rate can decrease from 21-76% with bilateral neurovascular bundle preservation to as low as 0-56% with unilateral nerve preservation [14-18]. After wide resection of both neurovascular bundles, recovery of spontaneous erections adequate for intercourse is rare[14-16].

Certain factors such as a serum PSA>10ng/ml, biopsy tumor Gleason >7, clinical stage T2a or higher, and a high number and percentage of biopsy cores involved with cancer, particularly with Gleason component 4 or 5 are believed to place a given individual at high risk for microscopic extraprostatic tumor extension[19]. Wide resection of the neurovascular bundle is necessary in these cases in order to achieve a negative surgical margin which is important to decrease the likelihood of subsequent biochemical recurrence [20]. The bilateral nerve sparing technique should be applied whenever possible. However in an unselected population of men with prostate cancer, a significant proportion will require unilateral nerve resection and rarely bilateral nerve resection to maximize the chance of cure. Even in patients undergoing bilateral nerve sparing radical prostatectomy, the post-operative potency takes time to recover and the rates are not 100%. It is conceivable that factors that improve nerve regeneration and growth could enhance earlier and more complete return of erectile function even in those men who undergo nerve sparing procedures. In addition to patients undergoing radical prostatectomy, neurovascular bundle damage can be sustained by individuals after radical cystoprostatectomy, low anterior colon resection and rectal surgery. The ability to preserve or replace these nerves could potentially increase the likelihood of post-operative potency in these patients as well[21]. This project is aimed as investigating biodegradable polymer conduits impregnated with laminin, schwann cells or neuronal stem cells as possible candidates for replacement of the cavernous nerve in an animal model.

BODY OF REPORT

Substrate fabrication: A chrome lithography mask was produced with the micron scale patterns of 10 μm groove width, and 20 μm groove spacing using conventional lithographic techniques. The mask was deposited onto a two-inch diameter quartz slide or a four-inch diameter silicon wafer in a vacuum chamber purged with argon at a pressure of less than 1 micro-Torr. Quartz substrates were etched up to 4 μm using deep reactive ion etching (DRIE) through the mask, leaving behind long rectangular areas capped by chrome. After the chrome was removed from the quartz substrate, the quartz substrate was used as a micro die to transfer the geometric microgrooves to the biodegradable polymer, poly(D,L lactide) (PDLA). A scanning electron microscopy image of the PDLA films is shown in Fig. 1. Laminin was selectively adsorbed to the microgrooves using a surface-tension based technique developed in our laboratory.

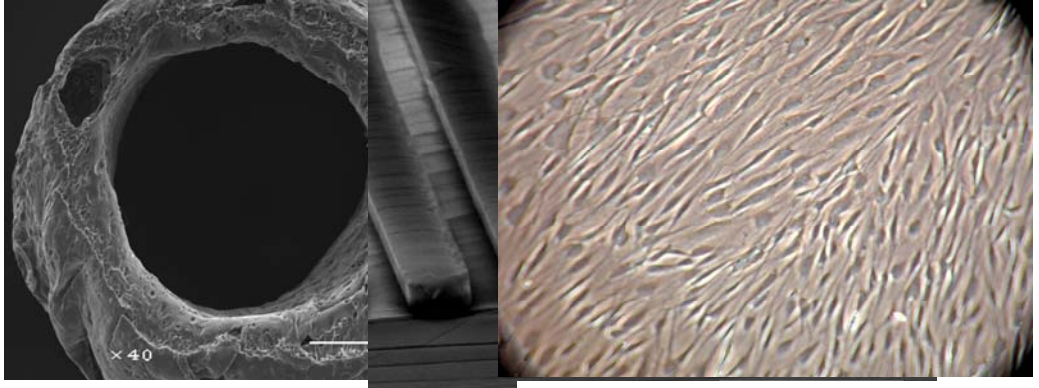

Fig. 3 SEM image of a PDLA co

Fig. 4. Schwann cells in culture

Fig. 1 SEM image of a PDLA micropatterned film

Conduit fabrication: PDLA was dissolved in chloroform at a concentration of 30% w/v. Sodium chloride crystals were ground, sieved with 120-gauge mesh, and suspended in the PDLA solution at a concentration of 75% and 50% by volume. A pasteur pipette (OD=1 mm) (Fisher) was dipped into 6% w/v solution of polyvinyl alcohol (PVA) in water and allowed to dry. The PVA acts as a release agent. The pipettes were then dipped in the polymer/salt suspension, slowly removed, and allowed to dry. This step was repeated until the outer diameter was 3mm. The dry conduits were placed under vacuum to remove any residual chloroform. The pipettes were placed in water to release the conduit from the support. Water was replaced every 2 hours until the sodium chloride was fully dissolved, leaving behind the conduit with 75% or 50% porosity. The conduits were dried, and cut into 1.2 cm sections. Depending on the salt concentration used, conduits with different porosities were obtained, as shown in Figs. 2 and 3. The micropatterned films were then cut into 1.2 cm by 1.5 cm sections, wrapped around flame narrowed pasteur pipettes, and inserted into the porous conduit supports. The conduits with micropatterned inserts were stored in desiccators at –20 °C until used.

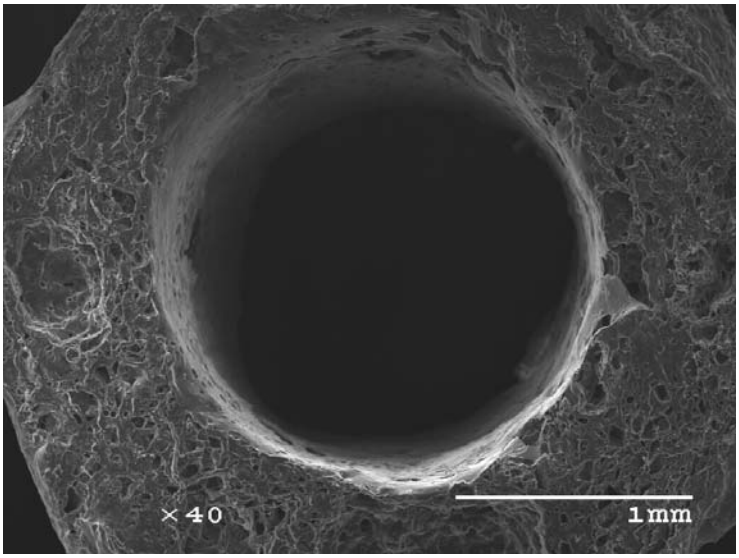

Fig. 2 SEM image of a PDLA conduit made using 50% sodium

Schwann Cell isolation and culture: Sprague-Dawley rats, 16-20 days old, were anesthetized and decapitated. The sciatic nerve was removed and kept in chilled Gey's Balance Salt Solution supplemented with 6.5 mg/ml glucose. The epineurium, connective tissue, and blood vessels were removed using fine forceps and the nerve was cut into pieces approximately 1 mm in length. The nerve pieces were placed in 60 mm tissue culture plates. Approximately 0.5 ml of chicken plasma solution (5 mg/mL) was spread evenly over the bottom of the plate. Nerve pieces were placed in the dish spaced 2 mm apart. One hundred microliters of thrombin (10 units/ml) was added to the plate and mixed with chicken plasma. Fibrin from the chicken plasma was allowed to clot for about five minutes before adding media. The media used for the cell cultures was DMEM with 10% v/v fetal bovine serum (FBS) and 5 μL gentamicin/ml medium .

Media was changed every two days. As the nerve pieces degenerated, fibroblasts began to spread onto the plate. When enough fibroblasts spread onto the plate in 5-7 days, the nerve pieces were transplanted onto new tissue culture plates using the above procedure. After 3 or 4 transplantations, the cells spreading from the nerve pieces consisted mostly of Schwann cells. At this point the nerve pieces were dissociated and then incubated for one hour at 37^0C. The media and cell debris were removed and the remaining cells will be resuspended in 1 ml of fresh media. The cell suspension was placed in 75 cm^2 tissue culture flask and enough media was added. Cultures were determined to be greater than 95% pure by immunocytochemistry staining of S-100 protein.

Schwann cell purification and seeding: Schwann cell cultures were purified by removing the cells from a 75 cm^2 T-flask and adding 2 mL anti-Thy 1.1 media to the Schwann cells for 30 minutes and adding 1.5 mL rabbit sera complement for another 30 minutes. The cells were resuspended and fed with DMEM/10% FBS supplemented with 0.5 mM forskolin, 0.5 mM isobutylmethylxanthate (IBMX) and 0.1 μg/mL human heregulin-β1 (EGF domain). This combination reduced fibroblasts while greatly enhancing the proliferation of the Schwann cells. Cells were not passaged more than 2 times to reduce chances of immortalization of Schwann cells. Schwann cell numbers were determined by trypan blue exclusion using a hemacytometer.

The Schwann cells were frozen down until required. Just prior to the *in vivo* studies, the Schwann cells cultures were thawed, allowed to become confluent, and were injected into each of conduit lumens 24 hours prior to surgery to allow for adhesion.

Animal studies

Following the in vitro studies, we have proceeded with the animal studies as planned. We originally obtained segments of cavernous nerve from several Sprague-Dawley rats and they were stored at -70^0C and shipped on dry ice to our collaborators (Dr. Mallapragada) at Iowa State University. Attempts at schwann cell extraction from these nerves were not very successful as the cells did not grow well in culture. We then resorted to using sciatic nerve derived schwann cells as described above.

For most of the animal experiments we obtained aged rates (retired male breeders). The rats were divided into groups by type of procedure/implant as follows:

1. Controls/sham surgery n=5
2. Nerve resection n=5
3. Laminin coated polymer tube grafts n=5
4. Genitofemoral nerve grafts n=5
5. Laminin coated polymer tubes plated with Schwann cells n=5

The animals underwent resection of 2mm of the cavernous nerve on either side after the nerve had been dissected out all the way to the pelvic ganglion. Following surgery/implantation, animals were observed for 2 months. All surgeries were performed under sterile conditions with ketamine/xylazine anesthesia initially followed by phenobarbitol anesthesia. An operating microscope with a 4x o 10x magnification was used for performing the surgery. The conduits as well as genitofemoral nerves were implanted using 10.0 nylon with a single suture at either end. The conduits (4mm long) were used as a sleeve to slide over the cut ends of the resected cavernous nerve. Laminin was applied to the conduits by injecting approx. 2mls of 10ug/ml solution (prepared in Earle's Balanced Salt Solution or PBS) into the middle of the conduit. Each tube was plated with 50,000 schwann cells. The Schwann cells were thawed and initially plated onto a T-25 flask with appropriate medium and used to plate the conduits after the second passage.

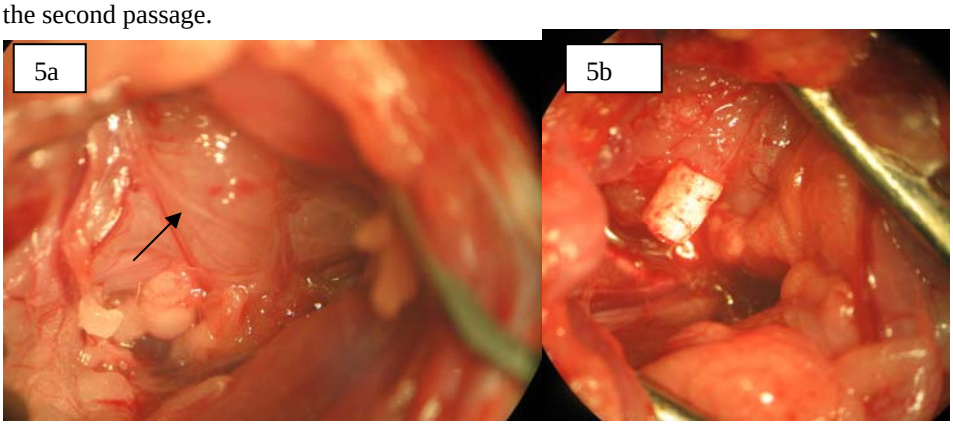

Fig. 5. Dissected cavernous nerve exiting pelvic ganglion in a rat shown by arrow (a).

Approximately one week prior to the 2 month time point two rats from each of groups 1-4 and three rats from group 5 underwent injection of fluorogold into the corpus cavernosum of the penis. All of these animals were sacrificed by phenobarbitol overdose one week later. We performed direct stimulation of the proximal end of the dissected cavernous nerve at the level of the pelvic ganglion to determine if we could elicit an erectile response from regenerated nerves. A Grass square wave generator was used as the power source and impulses at 4volts and a frequency of 20-60 Hz was delivered for 5-10 milliseconds. While no complete erections were observed, contraction of perineal musculature and penile tissue were observed visually in 60% of the animals. Following the electrical stimulation, cavernous nerve segments including the pelvic ganglion on each side were resected and submitted for histology. Immunofluoresence microscopy to assess for presence of fluorogold is currently pending. If the cavernous integrity has been restored or if there has been regrowth of the nerve, we expect to observe presence of fluorogold in the pelvic ganglion or proximal to the inserted nerve/conduit segment. Resected segment of cavernous nerve tissue from the other animals has been obtained and submitted for hematoxylin and eosin staining and will also be subjected to immunohistochemical analysis to look for nNOS expression using a polyclonal antibody to nNOS. Hematoxylin and eosin stained sections as well as unstained sections of tissue have been obtained and are awaiting staining for nNOS..

KEY RESEARCH ACCOMPLISHMENTS (related to statement of work):

Task 1:

To determine if biodegradable polymers alone or when impregnated with laminin +/- polylysine can result in nerve growth in vitro and can be used as a cavernous nerve interposition graft in vivo.

1. Production of micropatterned PDFA sheets and impregnation with laminin +/- polylysine and quantitate extent and direction of neurite growth (months 1-6) ***COMPLETED***
2. In vitro optimization of micropatterning to yield maximal directional neurite growth (months 7-12) ***COMPLETED***
3. Perform cavernous nerve interposition grafts using genitofemoral nerve and micropatterned polymer PDFA tubule graft alone or impregnated with laminin +/- polylysine in a rat model (month 7-8) ***COMPLETED***
4. Analyze return of cavernous nerve function in animals receiving no nerve grafts and those receiving nerve grafts using electrical stimulation, behavioral observation and nNOS expression (months 9-12). ***ELECTRICAL STIMULATION ANALYSIS COMPLETED. HISTOLOGIC ANALYSIS IN PROGRESS. BEHAVIORAL OBSERVATION COMPONENT MODIFIED TO USE PENILE BLOOD PRESSURE MEASUREMENTS AS THAT IS THE MORE COMMONLY USED METHOD CURRENTLY.***

Tasks 2-3: PLEASE NOTE THAT WE HAVE RECENTLY RESTARTED WORK ON THE PROJECT. SIGNIFICANT PROGRESS HAS NOTE BEEN MADE SINCE AUGUST OF 2005 DUE TO THE PI'S MOVE FROM THE UNIVERSITY OF IOWA TO THE UNIVERSITY OF CALIFORNIA SAN FRANCISCO. THE IACUC APPROVAL FOR THE ANIMAL PROTOCOL AT UCSF WAS RECEIVED ON 2/21/07 AND THE WORK HAS RESUMED. WE ARE CURRENTLY REVIEWING THE HISTOLOGY SLIDES WITH THE HELP OF A PATHOLOGIST (DR. LAURA TABATABAI) AND HAVE STARTED THE ANIMAL WORK. WE ANTICIPATE MAKING SIGNIFICANT PROGRESS IN THE NEAR FUTURE. WE APPRECIATE YOUR HELP IN OBTAINING THE NO COST EXTENSION.

CONCLUSIONS

We have able to successfully harvest and culture Schwann cells in vitro. We have also developed biodegradable polymer conduits that have been optimized for groove size to facilitate maximal directional neurite growth. We have been able to successfully implant the conduits and replace the cavernous nerve in experimental animals. We are awaiting results of histologic examinations to determine the adequacy of nerve regrowth and recovery of physiologic function which will be evaluated in the next phase of the experiments where animals will be examined after a longer period of recovery.

REFERENCES

1. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun M. Cancer statistics 2003. CA Cancer Clin. 53-26, 2003.
2. Seidman H, Mushinski MH, Gelb SK, Silverberg E: Probabilities of eventually developing or dying of cancer - United States, 1985. CA – Cancer J Clin 35:36-56, 1985.
3. Levine RL, Wilchinsky M: Adenocarcinoma of the prostate: a comparison of the disease in blacks versus whites. J Urol 121:761-762, 1979.
4. Catalona WJ: Nerve-sparing radical retropubic prostatectomy. Urol Clin N Am, 12:187-199, 1985.
5. Catalona WJ, Dresner SM: Nerve-sparing radical prostatectomy: extraprostatic tumor extension and preservation of erectile function. J Urol, 134:1149-1151, 1985.
6. Elder JS, Jewett HJ, Walsh PC: Radical perineal prostatectomy for clinical stage B2 carcinoma of the prostate. J Urol, 127:704-706, 1982.
7. Walsh PC: Radical prostatectomy, preservation of sexual function, cancer control: the controversy. Urol Clin N Am, 14(4):663-673, 1987
8. Walsh PC, Jewett HJ: Radical surgery for prostatic cancer. Cancer, 45:1906-1911, 1980.
9. Walsh PC, Mostwin JL: Radical prostatectomy and cystoprostatectomy with preservation of potency: results using a new nerve-sparing technique. Br J Urol, 56:694-697, 1984.
10. Rudy DC, Woodside JR, Crawford ED. Urodynamic evaluation of incontinence in patients undergoing modified Campbell radical retropubic prostatectomy: a prospective study. J Urol 132:708-712, 1984.
11. Walsh PC, Donker PJ. Impotence following radical prostatectomy: insight into etiology and prevention. J. Urol. 128:492, 1982.
12. Eggleston JC, Walsh PC: Radical prostatectomy with preservation of sexual function: pathological findings in the first 100 cases. J Urol, 134:1146-1148, 1985.
13. Steiner MS, Morton RA, Walsh PC: Impact of anatomical radical prostatectomy on urinary incontinence. J Urol, 145:512-514, 1991.
14. Quinlan DM, Epstein JI, Carter BS, Walsh PC. Sexual function following radical prostatectomy: influence of preservation of neurovascular bundles. J Urol, 145: 998,1991.
15. Geary ES, Dendinger TE, Freiha FS, Stamey TA. Nerve sparing radical prostatectomy: a different view. J Urol, 154:145-9, 1995.
16. Rabbani F, Stapleton AMF, Kattan MW, Wheeler TM, Scardino PT: Factors affecting recovery of erections after radical prostatectomy. J Urol, 164:1929-1934, 2000.
17. Catalona WJ, Carvahal GF, Mager DE, et al. Potency, continence and complication rates in 1,870 consecutive radical prostatectomies. J. Urol. 162:433-438, 1999.
18. Talcott JA, Rieker P, Propert KJ, et al. Patient reported impotence and incontinence after nerve sparing radical prostatectomy. J. Natl. Cancer Inst. 89:1117-1123, 1997.
19. Graefen M, Haese A, Pichlmeier U, Hammerer PG, Noldus J, Butz K, Erbersdobler A, Henke RP, Michl U, Fernandez S, Huland H. J Urol, 165:857-863, 2001.
20. Hull GW, Rabbani F, Abbas F, Wheeler TM, Kattan MW, Scardino PT. Cancer control with radical prostatectomy alone in 1000 consecutive patients. J Urol, 167:528-534, 2002.
21. Schoenberg MP, Walsh PC, Breazeale DR, Marshall FF, Mostwin JL, Brendler CB. Local recurrence and survival following nerve sparing radical cystoprostatectomy for bladder cancer: 10-year followup. J Urol, 155:490-494, 1996.

University of California
San Francisco

UCSF

Department of Urology
School of Medicine

April 10, 2007

1600 Divisadero Street
San Francisco, CA 94143-1695
fax: 415/885-7443

RE: Award #W81XWH-04-1-0038
Use of synthetic nerve grafts to restore cavernous nerve function following prostate cancer surgery: in vitro and in vivo studies.

Lisa Trott
Contract Specialist
USAMRAA, ATTN: MCMRC-AAA
Grants Transfer Section
820 Chandler St.
Ft. Detrick, MD 21702-5014

Dear Ms. Trott,

I am writing to request a no-cost extension period of 18 months and carryover of funds in the amount of $196,790 (see attached Expense Summary) for the above mentioned award. As you are aware, this award was transferred from the University of Iowa to the University of California San Francisco on July 6, 2006. However I did not commence working at the University of California San Francisco until August 8, 2006 and since then have been attempting to obtain and set up my laboratory as well as obtain final IACUC approval of my animal protocol.

The delay from the transfer and in IACUC approval being obtained have been the main reasons for this project being held up over the past 18 months. We have solely utilized funds for a small amount of my effort (5% from Aug. 06 through present), as I have spent at least this amount of time coordinating with various entities, trying to get everything in place for this project to proceed as proposed.

The subcontract with Iowa State University will be initiated once all protocols/approvals are in place.

I would greatly appreciate you considering this request and allowing us more time to perform the work. Please feel free to contact me if I can provide any additional information.

Best regards.

Badrinath R. Konety, MD, MBA
Associate Professor
Depts. Of Urology, Epidemiology and Biostatistics
University of California, San Francisco
tel: 415/353-7378
fax: 415/885-7443
email: bkonety@urology.ucsf.edu
www.ucsf.edu

I Concur:

Peter R. Carroll, Chair of Urology, UCSF

Joan Kaiser, Contracts & Grants Director, UCSF

IACUC Application Details AN075971-01

Summary	
Principal Investigator :	Konety, Badrinath
Title :	USE OF SYNTHETIC NERVE GRAFTS TO RESTORE CAVERNOUS NERVE FUNCTION FOLLOWING PROSTATE CANCER SURGERY: IN VITRO AND IN VIVO STUDIES
Purpose :	Research
Objectives :	Prostate cancer is the most common cancer in American males and is the second most common cause of cancer death. Curative therapy for prostate cancer involves surgery (radical prostatectomy RP) or radiation therapy. If radical prostatectomy is chosen as the treatment option it is important to resect the tumor completely. Positive surgical margins implying that residual tumor was left behind can be associated with a higher cancer recurrence rate and poorer survival. One of the complications of RP is the risk of damage to the cavernous nerves that are responsible for maintaining erectile function of the penis. In patients with large tumors, particularly if there is concern that cancer may extend beyond the capsule, the cavernous nerve(s) on that side are more likely to be damaged or even removed completely in an effort to completely excise the entire tumor. Replacement of a resected nerve with another nerve is one way to restore function to that nerve and has been successfully performed to restore function of the cavernous nerves and other nerves elsewhere in the body. There is a great interest in using this approach for replacing the cavernous nerves and such procedures have been performed in about 300 men to date. However, the results of such nerve replacements for the cavernous nerves are not uniformly good and in some instances removal of the donor nerves can itself result in loss of sensation or abnormal sensation in other parts of the body. We have developed synthetic biodegradable alternatives to function as nerve replacements for other nerves in the body and seek to investigate if these synthetic nerve replacements can be used to replace the cavernous nerve and restore erectile function. The hypothesis of this proposal is that replacement of the cavernous nerves using synthetic grafts can restore erectile function. The objectives will be to determine if synthetic nerve replacements impregnated with nerve growth promoting substances or cells can be used to successfully replace the cavernous nerve and can result in directional nerves that can be resected during prostate cancer surgery. The specific aims will be to determine the best type of synthetic nerve replacement and producing it in a laboratory and subsequently implanting them into rats. The success of these nerve replacements will then be assessed using electrical stimulation of the resected nerves and looking for substances expressed in tissue that would indicate regrowth of nerves. The studies in this proposal once translated to clinical practice will specifically be helpful to all patients undergoing radical prostate cancer surgery for treatment of their prostate cancer in avoiding one of the most frequent complications of such surgery, namely impotence. If these studies are successful this approach could be used clinically in the near future once FDA approval has been obtained. This may involve a time period of about 1-2 years. This approach could also benefit patients undergoing other types of surgery during which the cavernous nerves may be damaged such as surgery for rectal cancer and for bladder cancer. It is conceivable that covering the preserved nerves with these grafts containing nerve growth promoting substances can

	enhance potency rates even in those patients who do not have their nerves resected.
Status :	Preliminary Review In Progress
Expiration Date :	
Consulted LARC Vet :	Lawrence Carbone
Consult Date :	

Application History			
Project Number	**Approval Type**	**Approval Date**	**Expiration Date**
AN075971-01	New Approval		

A. Funding

Type:
Federal, State or Other Government

Funding Agency/Sponsor	Grant or Contract Number	Source has Approved Method for Scientific Merit Review
DOD	W81XWH-04-1-0038	Yes

You have provided a letter dated 9/24/2003 that refers to a different grant number. Provide a current grant award statement or letter for the correct grant number. Also send the "Vertebrate Animals" section of your grant application for a grant/protocol comparison.

You have selected at least one Project Funding Sources with an IACUC approved Method for Scientific Merit Review.

B. Regulated Materials and Stem Cell Info

Regulated Materials

Will data from this study be used to apply for Food and Drug Administration (FDA) approval of a drug or device, or will animal studies be outsourced?
No

Use of Human Embryonic Stem Cells:	
Will human embryonic stem cells or human somatic cells be used under this protocol?	No
Are the human embryonic stem cells on the NIH Human Embryonic Stem Cell Registry?	No
Does this protocol involve the transfer of a human somatic cell nucleus into an animal egg?	No
Does this protocol involve the combination of human embryonic stem cells with an animal embryo?	No

C. Animals

Species	USDA Type	Acquired	Bred	Total
Rat	D	140	0	140
	Total:	140	0	140

As required by federal regulations, describe the statistical tests (e.g. Power analyses) and/or other rationales (e.g. Tissue collection needs, breeding efficiency) that you used to determine the number of animals requested above. Note: The IACUC may require that you consult with a statistician from the UCSF Division of Biostatistics (476-8671).
Rationale for the number of animals used:
Minimal animal numbers will be used as determined by statistical analysis. Sample size calculations were based on

the chi square test for comparison of proportions and assumes an alpha = 0.05 and beta = 0.20 for a power (1-beta) of 0.80. Each of 7 series List these 7 groups here, with experimental variables as previously requested typically requires 20 rats per group. Therefore, a minimum of 140 animals are required to be included in the final analysis. Additional rats will be requested by modification if needed due to postoperative infection, self-inflicted wound emaciation, or death. We have sought to minimise the number of surgical complications by practicing microsurgical techniques on rats euthanized by other PIs.

There will be 7 groups with 20 rats / group. Group 1 will undergo sham operation and Group 2 will have nerve resection alone as negative controls. Group 3 will have autologous graft with the rat's native genitofemoral nerve. Group 4 will have a micropatterned tubule graft. Group 5 will have the tubule containing cultured Schwann cells implanted. Group 6 will have the tubule containing neuronal stem cells and Group 7 will have the tubule containing both Schwann cells and neuronal stem cells.

Will you be using any animals transferred from another PI or protocol, from your previous protocol, or transferred from another Investigator or Institution?

No

Will animals have undergone any procedures?

No

Describe the prior experimental procedures, justify the use of the animals for your research and submit a completed LARC Animal Transfer Form with your application or at the time of animal transfer.

Justify also if the animal(s) is/are used in more than one protocol involving a major operative procedure from which it is allowed to recover.

D. Contacts & Personnel

Name / *Role(s)	Degree	Title	Phone	Fax	Box	Email
Dahiya, Rajvir *Emergency Contact 2 *No Animal Contact	Ph.D.	Assoc Prof.	750-6964			rdahiya@urol.ucsf.edu
Konety, Badrinath *Principal Investigator *Emergency Contact 1	M.D.	Associate Professor	353-7171		1695	bkonety@urology.ucsf.edu
Lue, Tom	M.D.	Professor	476-1611	476-8849	0738	tlue@urology.ucsf.edu

Specify someone as the Alternate Responsible Individual.

Dahiya, Rajvir - *Functional Roles

***No Handling of Live Research Animals by this User.**

Add functional roles for Konety and Lue. Who is performing the procedures in this protocol?

Konety, Badrinath - Functional Roles

Species	Sup Ex	C&H	Anes	Surg	Post-Surg	Mon	Euth	Train

Lue, Tom - Functional Roles

Species	Sup Ex	C&H	Anes	Surg	Post-Surg	Mon	Euth	Train

If emergency euthanasia is required for an animal and the LARC vet staff is unable to contact the PI or any member of

their staff, please indicate which tissues/samples need to be collected from the animal, and specifically how they should be stored, e.g. Formalin, refrigeration, EDTA tube.
Prostate, cavernous nerves and implanted nerve grafts, penile biopsy tissue. To be stored in formalin.

E. Justifications and Alternatives

Sources			
Date of Most Recent Search	**Key Words**	**Search Site**	**Years Covered**
1/5/2007	nerve graft, nerve regrowth, pelvic nerve grafts, prostate cancer and nerve grafts, cavernous nerve regeneration, Schwann cell regeneration	PubMed	1966-2006
1/5/2007	nerve grafts, nerve regrowth, prostate cancer and nreve grafts, cavernous nerve grafts, Schwann cell regeneration, perlvic nerve regrowth	Altweb, CRISP, Biomedical Research Database (1998-2001), FEDRIP	

Carbone: I'd revisit the search including the keywords "animal models"

Other Resources		
Date	**Topics**	**Resource (e.g. Attendance at meetings, consultation w/ colleagues)**
Move these searches up to the previous section1/5/2007	nerve grafts, nerve regrowth, prostate cancer and nreve grafts, cavernous nerve grafts, Schwann cell regeneration, perlvic nerve regrowth	Altweb, CRISP, Biomedical Research Database (1998-2001), FEDRIP

Explain why animals are required for your studies, and why replacements, such as cell culture or computer modeling, cannot fully replace animals.
Non-animal replacements such as cell culture would not suffice for this study as we are studying anatomical structures which correspond to human anatomy, as well as their in vivo function. Computer models can be used to identify optimal structure for conduits and possibly project rate of growth of nerve endings, but in vivo data is required before this method of nerve replacement can be applied to humans

The nerve growth implants have already been tested in vitro and therefore an in vivo model is required before the technique can eventually be applied to humans. We require the animal model testing since the experiments are designed to assess if the nerve grafts implanted during the studies can elicit physiologic function in vivo.

Explain why the proposed species are the most appropriate.
Species identification and rationale:
Sprague-Dawley rats (male) – most previous studies in this area have used this rat as a model. The pelvic nerve is easily identifiable and detectable for surgical manipulation. Research on erectile function have used this model and the methods of assessing erectile function are most well established in this animal.

Describe how your proposal minimizes animal pain and distress (e.g. use of in vitro procedures, reduction of animal numbers, refinement of experimental design, refinement of procedural techniques). Please be specific.
Alternatives to painful procedures:
We searched the Altweb database as well as Medline for alternatives to the procedures described in this protocol. Searches were conducted using the keywords 'nerve grafts, 'pelvic nerve, 'erectile function", from the years 1966-present (Medline); all available records (Altweb), date of search was 1/5/2007. No other alternatives to the survival surgery proposed in this protocol were found. We will utilize sterile instruments and aseptic techniques for all survival surgery. Animals will be anesthetized prior to the surgery, and analgesics (buprenorphine) will be administered post-operatively. The surgery involves a very small incision Sect. G says "incision from xiphoid to symphysis pubis," which is not a small incision for

the rat which will be closed and minimal manipulation of intra-abdominal tissues. We have reduced animal numbers to the minimum necessary and we have completed all the in vitro procedures we can and need to evaluate our results in an in vivo setting at this time. Previous in vitro experiments have focused on guiding and accelerating peripheral nerve growth using a combination of physical, chemical and cellular cues on biodegradable polymer substrates. Techniques have been developed for patterning biodegradable polymer substrates and selectively attaching proteins such as laminin in the grooves. The microgrooves act as physical guidance channels and the presence of laminin in the grooves coaxes the axons to grow along the grooves. Schwann cells pre-seeded in the grooves secrete nerve growth factor and accelerate neurite alignment in the direction of the grooves. The studies were conducted with rat dorsal root ganglia (DRG) and Schwann cells from rat sciatic nerves. These methods have also been extended to neuronal stem cells. We have determined that biodegradable polymers impregnated with nerve growth promoting substances or neuronal stem cells and glial cells can be used as interposition nerve grafts for the cavernous nerve and can result in directional nerve growth in vitro. At this point it is necessary to test our hypothesis in vivo in an animal model.

You do not have USDA Category C Animals.

You have USDA Category D Animals that would receive relief from pain, discomfort or distress.

You do not have USDA Category E Animals.

F. IACUC/LARC Standard Procedures

Rat
Standard Procedures

Metal ear tags
Rodent Identification.
Tissue collection from animals euthanized under this protocol only (must adhere to the 2000 AVMA Panel on Euthanasia, 6th Edition).

Note: ONLY those techniques listed above are IACUC/LARC Standard Procedures. You must follow the procedures as specified or you will be out of compliance. Any variation must be described and justified below, and approved by the IACUC.

G. Procedures Involving Living Animals

Species: Rat Group: D

There will be 7 groups with 20 rats / group. Group 1 will undergo sham operation and Group 2 will have nerve resection alone as negative controls. Group 3 will have autologous graft with the rat's native genitofemoral nerve. Group 4 will have a micropatterned tubule graft. Group 5 will have the tubule containing cultured Schwann cells implanted. (Any concern with graft rejection if these are not autologous grafts?. Group 6 will have the tubule containing neuronal stem cells and Group 7 will have the tubule containing both Schwann cells and neuronal stem cells. Tissues for cells and grafts will be supplied from the University of Iowa from the cultures of a collaborator (Dr. Mallapragada).delete names in the text

The PI, who is a urologist, has extensive experience in microdissection techniques in humans and animal models and will be performing most of the dissections. It is anticipated that in the future a technician, who will be added to this protocol, will be hired and trained to assist and perform the nerve graft procedures. The PI also has experience performing cavernous nerve grafts in humans.

Outcome from the nerve replacement procedure will be assessed in half the rats at 2 months and in the other half at 4 months following the procedure. Both physiologic functional and tissue level biochemical assessment will be

performed. The animals will be tested for physiologic function complete as described below.
Technical methods (animal procedures):

1) Anesthesia- Rats will be initially weighed. Rats will then be given an intraperitoneal injection of a combination of ketamine (90 mg/kg) and Xylazine (10 mg/kg). The rats will then be placed on a heating pad to maintain core body temperature of 37 degrees celciuscelsius. Carbone: I would consider adding a local block (lidocaine and / or bupivicaine), esp. if you're really making incision all the way from xiphoid to symphysis pubis. Also, consider titrating ketamine-xylazine depth with isoflurane if initial injection does not have them at the right plane for you.

2) The abdomen will be shaved with an electric razor and surgically prepped with poviodine surgical prep solution. A midline incision from xiphoid to symphysis pubis will then be made and the intra-abdominal contents and testes retracted upwards in the wound to expose the prostate and perirectal space. The pelvic ganglia will then be identified on either side of the rectum using microdissection. The cavernous nerve exiting from the inferior aspect of the ganglia will be identified.

3) A 5mm segment of the nerve will then be resected in animals destined for treatment or negative control groups while the sham operation animals will undergo primary reclosure of the wound.

4) The anastomosis for all grafts and tubules will be performed using 10-0 nylon suture microscopically. The autologous graft if used, will be about 1.5X the distance of the gap and reversed in orientation to allow for condensation of nerve fibers at the distal end. The abdomen will be closed in two layers with running chromic suture and the skin will be reapproximated with staples. These will be left in until time of sacrifice justify leaving sutures in "until the time of sacrifice" – IACUC standard precedures require suture removal 10-14 days post-op

5) Animals will be monitored until recovered from anesthesia and returned to their cages. Prior to waking the rat a dose of buprenorphine will be given (0.03-0.05 mg/kg subcutaneously)

6) One week prior to evaluation of physiologic function 100 microliters of fluorogold will be directly injected into the penile tissue of all the rats to be assessed whilehere you are "gently restraining the awake animals"for this injection gently restraining the awake animals. But in the next sentence you say "Animals will be sedated with isoflurane prior to injection" Animals will be sedated with isoflurane prior to injection. Evaluation of physiologic function will occur in half of the animals after 2 months, and in the other half after 4 months.

7) To assess physiologic function, animals will be anesthetized with isoflurane inhalation and the abdomen re-entered through the previous incision. The penis will be circumcised and the penile skin will be dissected out to expose the corporal bodies. The pelvic ganglia will again be identified on either side of the rectum using microdissection.

8) The cavernous nerve proximal to the resected and replaced segment will then stimulated using bipolar 32 gauge steel electrodes (TECA, Oxford Instruments, New York). The positive electrode will be placed 3-4mm cephalad to the negative electrode. The stimulation will be performed using a Grass Square wave stimulator S-48 (Grass Instruments, Quincy, MA) with a 4-volt stimulus being administered at a frequency of 20 Hz for 5-10 milliseconds. Occurrence of erection will be visually documented and assessed. Any change in girth and/or length of the penis will be recorded as a positive response. Only a presence or absence response will be recorded for erections since assessment of the quality of the erections using gradations of response will be subjective.

9) In addition, penile blood pressure measurements will be performed by placing a 21 gauge needle in the penile body and determining pressure via a transducer. Reference blood pressure measurements will be performed by cannulating the carotid artery through a cut-down procedure and placing an 18 gauge needle or a 5cm length PEG tubing attached to a pressure transducer into the artery and held in place by a silk ligature.

10) Animals will then be euthanized with a Nembutal overdose and bilateral thoracotomy following assessment of erectile function. The cavernous nerve will then be harvested for histological examination. Biopsies of penile tissue will also be obtained prior to euthanasia by directly excising 3 small portions of penile tissue using scissors or scalpel. The tissues will be placed in formalin and later assessed by histochemistry for presence of iNOS through immunostaining.

Anesthesia/analgesia/tranquilization:

Anesthesia used – ketamine (90 mg/kg) and Xylazine (10 mg/kg). above you said isoflurane

Analgesia used - Buprenorphine 0.1-0.25mg/kg PO TID as needed or Butorphanol 2mg/kg SQ Q4H as needed Carbone: Current thinking/studies suggest that oral buprenorphine is unreliable in rats. My recommendation would be to give two subcutaneous buprenorphine doses separated by 8-12 hours AND 1 -2 doses of an NSAID (carprofen or meloxicam), the firs the day of surgery, the next the following morning.

H. Surgery and Post-Operative Care

Rat	
Surgery Performed:	Yes

Surgery Type:	Survival (Single)
What is the duration of the surgery?	20 - 30 minutes
For how long will the animals survive after surgery?	2 - 4 months
Describe post-operative care and the frequency of monitoring in the days or weeks until the animals recover from the surgery (e.g. wound care, infection, etc.)	Animals will be monitored post-surgery for recovery from anesthesia and will then be placed back in their cages and given analgesics as needed. They will be checked once a day for 3 dayshere you say daily for 3 days, but in Sect. J you say daily for one week then once a week till end of study period (2 or 4 months depending on group) to look for wound infection, altered feeding and watering habits and ambulation.
Will you follow IACUC/LARC Guidelines for Post-Operative Analgesia:	Yes
Will you follow IACUC/LARC Guidelines for Animal Surgery:	Yes

I. Pre-Anesthetics and Anesthetics, Neuromuscular Blocking Drugs, Therapeutics, Analgesics and Experimental Agents

Rat: Pre-Anesthetics and Anesthetics						
Agent	**Dose Range (mg/kg)**	**Route**	**Frequency / Total Duration**	**Recover From Agent**	**Use IACUC Monitor Form**	**Anesthesia Recovery Time**
Ketamine + Xylazine; May include partial reversal with Atipamezole Add isoflurane to this table (as mentioned in sect. G)	75-100 Ketamine + 5-10 Xylazine; 0.1 - 1.0 Atipamezole	Intraperitoneal combined in one syringe. Aptipamezole may be administered intraperitoneally or subcutaneously	Every time animal undergoes anesthesia; redose as needed	Yes	Yes	60-90 minutes

Rat: Pre-Anesthetics and Anesthetics Monitoring Details					
Agent	**Variable Monitored**	**Monitoring Frequency [Anesthesia]**	**Monitoring Frequency [Recovery]**	**Doc/Charting Frequency [Anesthesia]**	**Doc/Charting Frequency [Recovery]**

You have omitted this section. Add parameters to be monitored during anesthesai by clicking on "monitoring details."

Rat: Therapeutic			
Agent	**Dose Range (mg/kg)**	**Route**	**Frequency / Total Duration**
Buprenorphine (Buprenex) Carbone: please see my notes above, about giving each rat 2 doses of subcutaneous buprenorphine and 2 doses of an NSAID, and possibly some local analgesic at incision.	0.03-0.05 mg/kg	subcutaneously	Prior to waking from anesthetic and prn for pain, lethargy, and lack of appetite. These parameters will be monitored as in section J2 (Adverse Effects)

Complete the table for Experimental agents. List all agenst to be administered to the rats, including the nerve grafts, cells, and growth factors, and the fluorogold injected into the penis

All Species: Pre-Anesthetics and Anesthetics	
Will you follow IACUC/LARC Guidelines for Anesthetizing Animals for Research Procedures:	Yes

J. Management and Monitoring of Adverse Effects of Procedures and Experimental Agents

Adverse Effects: Rat Experimental Group: D		
Procedure, Agent or Phenotype	**Potential Adverse Effects**	**Management**
woundsurgery	hematoma	Pressure to wound, or reexplore
woundsurgery	infection	provide antibiotics
Cavernosal nerve injury	impotence	nothing. this is expected
Pain Nerve grafts	Pain Below you mention the possibility of graft rejection. What are the signs of graft rejection that would lead to euthanasia?	Treat with buprenorphine (dosage listed above) euthanasia

If the incision is long, dehiscence is a realistic outcome to plan for.

Monitoring Parameters: Rat Experimental Group: D		
Monitoring Parameters	**Frequency make this consistent with Sect. H. Also indicate how often you will see the rats after 2 weeks, since they will survive for 2 or 4 months**	**PI/Lab will Document**
respiratory rate	Once a day for 1 week then once every 2 days for 2 weeks	Yes
respiratory pattern	Once a day for 1 week then once every 2 days for 2 weeks	Yes
eating behaviorweight is a better indicator of rat health than "eating behavior" (and you must weigh the rats since below you are using 15% weight loss as a criterion for euthanasia)	Once a day for 1 week then once every 2 days for 2 weeks once a week is probably enough for weighing after the first week	Yes
Socializing	Once a day for 1 week then once every 2 days for 2 weeks	Yes
Wound care	Once a day for 1 week then once every 2 days for 2 weeks	Yes
Vocalizing	Once a day for 1 week then once every 2 days for 2 weeks	Yes

Describe the conditions, complications, and criteria (e.g. uncontrolled infection, loss of more than 15% body weight, etc.) that would lead to removal of an animal from the study, and describe how this will be accomplished (e.g. stopping treatment, euthanasia).

Animals would be removed form this study due to uncontrolled infection, loss of greater than 15% of body weight, or rejection of the nerve graft. These animals would be euthanized by nembutol overdose and bilateral thoracotomy.

For all investigators housing animals with tumor formation, skin lesions, neurological deficits, or that are in Category E, list the expected characteristics/clinical presentations and endpoints of the animal model and the criteria for euthanasia. Note: The IACUC also requires such lists to be posted in the respective animal rooms and monitored by the IACUC compliance staff and LARC, to assure PI adherence to the endpoints listed.

K. Species Locations

Rat - "LARC Space" Locations
Use LARC Space for Animal Housing of this species.
Use LARC Space for Extended Study of this species.

Use LARC Space for Survival Surgery on this species.

Rat - "Transporting"
You will NOT be transporting animals in your own vehicles.

L. Reportable Exceptions for Procedures

Rat

M. Physical Restraint of Conscious Animals

Rat	
Number of animals that will be restrained:	None

N. Euthanasia

Rat	
Will you conform to the UCSF Guidelines for Euthanasia?	Yes

Chemical Method	Physical Method	Comments
Anesthetic overdose (specify)	Bilateral thoracotomy	Rats will undergo bilateral thoracotomy under nembutol anesthesia

O. Environmental Enrichment

Rat	
Will you conform to the UCSF Guidelines for Environmental Enrichment?	Yes

P. Tissue Sharing and Live Animal Disposition

Rat	
Name and telephone number of the contact person to discuss tissue-sharing arrangements:	
Contact Name:	Badrinath Konety
Contact Telephone:	415-353-7378
Live Animal Disposition	
Describe your plan, if you are willing to make live animals available after your study:	N/A

Rat - Available Tissues						
Strain	Gender	Age	Weight	Tissues Not Available	Form of Euthanasia	Tissue Alterations

Q. Roles & Training

Dahiya, Rajvir - Details	
Admin Role :	Emergency Contact 2,No Animal Contact
CDP Questionnaire :	NOT Completed
BRER I Training :	Course NOT Taken; BRER I course is required for all IACUC Personnel [every 3 years].
BRER II Training :	Course NOT Required; BRER II course is required for IACUC Personnel involved with Anesthesia, Surgery and/or Post Surgical Care [every 3 years].

Dahiya, Rajvir - Training Records	
Course Title	**Date**

Konety, Badrinath - Details	
Admin Role :	Principal Investigator,Emergency Contact 1
CDP Questionnaire :	Completed
BRER I Training :	Course NOT Taken; BRER I course is required for all IACUC Personnel [every 3 years].
BRER II Training :	Course NOT Required; BRER II course is required for IACUC Personnel involved with Anesthesia, Surgery and/or Post Surgical Care [every 3 years].

Konety, Badrinath - Training Records	
Course Title	**Date**
IACUC - BRER II (Online)	10/11/2005
Laboratory Housing (Online)	10/11/2005

Lue, Tom - Details	
Admin Role :	
CDP Questionnaire :	Completed
BRER I Training :	Course Taken; BRER I course is required for all IACUC Personnel [every 3 years].
BRER II Training :	Course NOT Required; BRER II course is required for IACUC Personnel involved with Anesthesia, Surgery and/or Post Surgical Care [every 3 years].

Lue, Tom - Training Records	
Course Title	**Date**
AWAP - BRER Update	11/21/2002
AWAP - Mouse: exempt	12/31/1998
AWAP - Rabbit: Exempt	12/31/1998
AWAP - Rat: Exempt	12/31/1998
AWAP - Rat: Exempt	12/31/1998
IACUC - BRER I (Online)	09/17/2006
IACUC - BRER I (Online)	07/28/2003
IACUC - BRER II (Online)	09/17/2006
IACUC - BRER II (Online)	10/13/2003
Laboratory Housing (Online)	02/03/2005
PSB Classroom Presentation	02/28/2005
PSB Facility Orientation	02/28/2005

Expenditure Summary By DPA, Fund, NCA Group for 03/2007 (CLOSED)
Run date - 04/12/2007 09:30

Grouping	Budget	Financial	Lien	Balance	Pct
DPA - 444958 MED RES-UROLOGY					
Fund/FY - 23115-01 W81XWH-04-1-0038					
- DIRECT					
- SALARY					
ACADEMIC	48,115.00-	8,250.03	0.00	39,864.97-	17.15%
Total:	48,115.00-	8,250.03	0.00	39,864.97-	17.15%
- BENEFITS					
ACADEMIC	10,995.00-	858.92	0.00	10,136.08-	7.81%
Total:	10,995.00-	858.92	0.00	10,136.08-	7.81%
- NONPAYROLL					
UNALLOCATED	4,480.00-	0.00	0.00	4,480.00-	0.00%
OTHER SERVICES	14,211.00-	0.00	0.00	14,211.00-	0.00%
TRAVEL	2,500.00-	0.00	0.00	2,500.00-	0.00%
SUBAWARD CUM EXP<=$25,000	25,000.00-	0.00	0.00	25,000.00-	0.00%
SUBAWARD CUM EXP>$25,000	50,449.00-	0.00	0.00	50,449.00-	0.00%
CAMPUS UNALLOCATED	0.00	0.00	0.00	0.00	N/A
Total:	96,640.00-	0.00	0.00	96,640.00-	0.00%
Total:	155,750.00-	9,108.95	0.00	146,641.05-	5.85%
- INDIRECT					
- OVERHEAD					
OVERHEAD	54,919.00-	4,873.32	0.00	50,045.68-	8.87%
Total:	54,919.00-	4,873.32	0.00	50,045.68-	8.87%
Total:	54,919.00-	4,873.32	0.00	50,045.68-	8.87%
Total:	210,669.00-	13,982.27	0.00	196,686.73-	6.64%
Total:	210,669.00-	13,982.27	0.00	196,686.73-	6.64%
Totals:	210,669.00-	13,982.27	0.00	196,686.73-	6.64%

Selected Report Criteria
Business Unit: UCSF
Fund Type: Current
Fund: 23115*
Date: 03/2007